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TO

OFFICE OF NAVAL RESEARCH
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BIOLOGICAL SCIENCES DIVISION
CLINICAL BRANCH
LT. COMMANDER PAUL LINDSAY, HEAD

FROM

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"PLANT COLLOIDS FOR EVALUATION AS PLASMA EXTENDERS
AND FOR COMPLEXING WITH PROTEIN SPARING SUBSTANCES"

REPORT NO. NINE

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Synthetic Blood Plasma Extenders

Introduction

Work was continued during January on the preparation of pectate esters for use as plasma extenders. Particular emphasis was placed upon the synthesis of glycerol pectate. Preliminary attempts were also made to esterify pectic acid with sorbitol anhydride.

Summary

Several possible methods to synthesize glycerol pectate are presented herein. Among these, the reaction of sodium pectate with glycerol α -monochlorohydrin and that of pectic acid with glycidol were thus far investigated.

Attempts to prepare glycerol pectate by reacting sodium pectate with glycerol α -monochlorohydrin in water or water miscible organic solvents, were unsuccessful. The reason for this failure was assumed to be the fact that no solvent was available in which sodium pectate and glycerol pectate were soluble, but in which sodium chloride was insoluble. Removal of sodium chloride by precipitation, or by some other means, was felt essential to drive the reaction in the desired direction.

Esterification studies of pectic acid with glycidol lead to the successful preparation of glycerol pectate under conditions very similar to those elaborated for 2-hydroxypropyl pectate by reacting pectic acid with propylene oxide. The presence of inorganic salts, as demonstrated with NaCl, inhibited the ester formation. Sodium pectate and glycerol α -monochlorohydrin were believed to have been obtained instead. In comparison with 2-hydroxypropyl pectate, glycerol pectate showed decidedly greater water solubility. With regard to relative viscosity and oncotic pressure there was no outstanding difference between the two derivatives.

A small batch of glycerol pectate was prepared and submitted to Dr. F. W. Hartman, Henry Ford Hospital Research Laboratories, for evaluation in vivo. According to Dr. Hartman's studies on mice and rabbits, glycerol pectate showed no signs of acute toxicity. Upon his request, larger amounts of this material are being prepared for acute bleeding tests on dogs.

Preliminary attempts were made to prepare sorbitol pectate from pectic acid and sorbitol anhydride.

Experimental

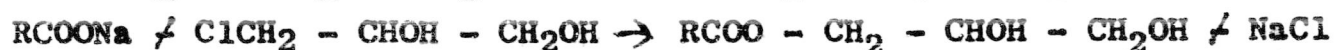
A. Glycerol Pectate

In attempting to synthesize glycerol pectate, the following routes were considered.

- 1) Direct esterification of pectic acid with glycerol.
- 2) Reaction of pectate salts with glycerol α -monochlorohydrin.
- 3) Esterification of pectic acid with glycidol (2,3 - epoxy-1-propanol).
- 4) Transesterification

Route 1 was ruled out for obvious reasons. The high temperatures and the presence of an acidic catalyst, as customary in direct esterification of free acids, were expected to degrade the pectic acid molecule.

Route 2 appeared more promising provided a solvent could be found in which the glycerol pectate were soluble but not the inorganic salt, i.e., NaCl formed by reacting sodium pectate with glycerol α -monochlorohydrin:



It was unpredictable whether this reaction could also be achieved in an aqueous medium.

In analogy to the successful esterification of pectic acid with ethylene or propylene oxides to the glycol and propylene glycol pectate, route 3 appeared the most promising approach. The main difficulty to be overcome appeared to be the instability of glycidol, as previous attempts showed it to polymerize very readily.

Transesterification has not yet been attempted but will be the subject of future studies.

1. Reacting Sodium Pectate with Glycerol α -Monochlorohydrin

A great number of attempts were made to obtain glycerol pectate by reacting sodium pectate with glycerol α -monochlorohydrin. The starting material was commercial Pectic Acid #75, which was directly weighed into 4 ounce screw cap bottles. The pectic acid was then suspended in the appropriate solvent and neutralized by adding an equivalent amount of NaOH solution. Upon adding glycerol α -monochlorohydrin the bottles were incubated at 75°C, and shaken at frequent intervals. In following the progress of esterification, samples were withdrawn to which, upon diluting with water, an aqueous solution of calcium chloride was added. Where glycerol pectate was formed, a calcium pectate precipitate should be absent.

The following table represents a selection of some of the more typical charges investigated.

Run #	Pectic Acid g	meq	Chlorohydrin g	meq	H ₂ O ml	Dioxane ml	Pyridine ml	Formamide ml
127-1	2	7.5	2	18	7.5	25	---	---
127-2	2	7.5	2	18	15	---	---	---
127-7	2	7.5	4	36	15	---	5	---
140-24	4	15	2	18	31	---	---	---
140-25	4	15	2	18	5	---	---	25

None of these attempts gave even the slightest indication that esterification had occurred. Negative results were also obtained by reacting free pectic acid with glycerol α -monochlorohydrin in the presence of an excess of pyridine.

The failure of the experiment just described, may be attributed to the fact that the solvents for the reactants were not selective for either the glycerol pectate or NaCl. If a solvent were available in which NaCl is insoluble, the desired reaction is more likely to occur, as the NaCl would then be eliminated as fast as it is being formed. Of a number of organic solvents tested so far, only formamide proved a fair solvent for pectate esters. Free pectic acid and pectate salts, however, were insoluble in formamide.

2. Reacting Pectic Acid with Glycidol

(a) Studies with Purified Glycidol

Three moles of glycerol α -monochlorohydrin dissolved in 15 moles isopropanol were reacted with 3 moles NaOH, as previously described. Sodium chloride (155 grams) was isolated with a yield of 89%. The crude glycidol concentrate was then subjected to vacuum distillation. After removal of isopropanol and water, glycidol distilled within the range of 51-52°C. at 9-10 mm Hg with a bath temperature of 80°C. Yield based on chlorohydrin: 30%.

The following experiments were carried out with distilled glycidol in 4 ounce bottles at room temperature.

Sample	Pectic Acid g	Glycidol ml	H ₂ O ml
137-16	4	10	20
137-18	4	12	28
137-17	4	10	1.6

After shaking over night (16 hours), highly viscous solutions resulted with samples #137-16 and #137-18. Addition of calcium chloride to these syrups, diluted with water, no longer precipitated calcium pectate. Evidently esterification of pectic acid was accomplished. The slight acid reaction observed at 16 hours had completely disappeared after a total reaction time of 40 hours. These findings demonstrated that the formation of glycerol pectate from pectic acid and glycidol proceeded at about the same rate and to the same extent as that of propylene glycol pectate from pectic acid and propylene oxide.

Bottle #137-17 was charged with a ratio of glycidol and water to pectic acid comparable to that previously employed in the heterogeneous esterification procedure for 2-hydroxypropyl pectate. Tests performed at 16 and 40 hours reaction time, however, showed no apparent esterification. The reason for this difference in behavior remains to be explained. Future attempts to esterify pectic acid in a heterogeneous system will be made at slightly higher temperatures.

(b) Attempted Esterification with Crude Glycidol

Crude glycidol was prepared in a 2-liter resin reaction kettle provided with agitator, separatory funnel and thermometer. Two moles (221 grams) of glycerol α -monochlorohydrin-(Eastman) dissolved in 10 mole% isopropanol (765 ml) were cooled to -5°C . Over a period of one hour a solution containing 80 grams of NaOH, dissolved in 80 ml H₂O, was gradually added, and the temperature was maintained at -5°C . Agitation was continued for two more hours to complete the reaction. The NaCl formed was removed by filtration, rinsed with isopropanol and dried at 100°C to constant weight. Yield: 95 grams, equivalent to 31% conversion.

The filtrate containing the glycidol was evaporated at reduced pressure (about 20 mm Hg) in a water bath kept at $25-35^{\circ}\text{C}$. A light viscous, amber concentrate (165 ml) was obtained reacting slightly alkaline. Ten ml of this concentrate were calculated to contain approximately 1/10 mole of glycidol.

Using this concentrate, esterification of pectic acid was attempted in 4 ounce screw cap bottles with the following charges:

Run No.	T °C	Pectic Acid		Glycidol Conc.		H ₂ O
		g	meq	ml	meq	ml
132-8	70	4	15	10	100	---
132-9	22	4	15	5	50	---
132-11	22	4	15	10	100	---
132-12	22	4	15	10	100	10

The bottles were shaken for 48 hours without showing any indication of reaction. The samples were then diluted with water and filtered. The filtrates contained a small amount of pectic substances precipitable with acetone. The precipitates when redissolved in water as well as the filtrates themselves, yielded insoluble calcium pectate upon addition of calcium chloride. Consequently, a small amount of sodium pectate must have been present in the filtrates.

The solids removed by filtration proved to be chiefly unchanged pectic acid. Therefore, esterification had not taken place.

A check on the glycidol concentrate revealed a slight alkalinity, assumed due to a trace of free NaOH. The free alkali was expected to neutralize a fraction of the pectic acid yielding sodium pectate which could no longer be esterified. In order to rule out this possibility, esterification attempts were made with neutralized, and also slightly acidified glycidol concentrate. The individual charges are given in the following table.

Run No.	Pectic Acid	Glycidol Conc.	H ₂ O	1-N H ₂ SO ₄
	g	ml	ml	ml
134-13	4	20	10	3
134-14	4	20	15	5
134-15	4	20	10	15

After shaking these samples for three days at room temperatures, esterification could still not be achieved. There remained only one possible explanation for the failure of the esterification, namely, the inhibition by NaCl known to be present in the glycidol concentrate. This was shown to be the case as demonstrated on an experiment described in paragraph (c).

(c) The Inhibition of the Glycerol Pectate Formation by NaCl

The following experiment was conducted to answer the question whether NaCl, or inorganic salts in general, could inhibit the formation of glycerol pectate from pectic acid and glycidol.

A bottle was charged with 2 grams (≈ 7.5 meq.) Pectic Acid #75, 10 ml H_2O , 5 ml distilled glycidol, and 0.6 grams (10 meq.) NaCl. With the exception of NaCl (present in slight molar excess to pectic acid) the charge was thus the same as sample #137-16 yielding complete esterification as mentioned in paragraph (a).

The bottle was shaken at room temperature for several days without ever getting a homogenous viscous solution. When finally examined, the solids were found water soluble and the heterogenous reaction mixture, when diluted with water, reacted neutral. From this solution calcium pectate was immediately precipitated on addition of $CaCl_2$. Thus, it appeared that sodium pectate was obtained instead of glycerol pectate and that glycidol reacted with HCl to glycerol monochlorohydrin, the proposed reaction being the following:



The probability of such reaction mechanism is emphasized by the fact that pectic acid has known ion exchange properties, with a relatively strong affinity for inorganic cations, and the glycidol in turn very easily adds HCl to give glycerol α -monochlorohydrin. Based upon these considerations, the failure to obtain glycerol pectate by reacting pectic acid with crude glycidol containing NaCl is therefore readily explained.

3. Preparation of Glycerol Pectate Lot No. 1

Described below is the first attempt to prepare glycerol pectate from pectic acid and glycidol in somewhat larger amounts for preliminary studies in vivo. Glycidol was prepared in the usual manner by reacting glycerol α -monochlorohydrin (4 moles) at low temperature. The chlorohydrin used herein was obtained from Lack & Company, and was 93% pure. It differed from the earlier material by being completely colorless, and being free of acid impurities. Based upon the isolated NaCl, a 94.5% conversion was achieved.

The glycidol concentrate was then subjected to vacuum distillation, and pure glycidol (191 grams) was obtained in 66% yield of Bp. 48-50°C. at 7mm Hg.

A 32 ounce beverage bottle was charged with 70 grams-Pectic Acid #75, 188 grams glycidol and 350 ml H₂O, and tumbled end-ever-end in a bath maintained at 40°C. After 16½ hours a dark brown slightly foaming syrup was obtained which on diluting with water showed neutral reaction. No precipitation could be detected when calcium chloride was added. Thus, complete esterification of pectic acid was indicated.

The crude glycerol pectate was then bleached by treating with 250 ml ClO₂ solution for 22 hours. The bleached reaction mixture was diluted with water to one liter and filtered with 1% Dicalite Special Speedflow. Glycerol pectate was precipitated from the filtrate (1200 ml including rinse) by adding an equal volume of acetone. The supernatant liquid was decanted, and the solids were rinsed by slurrying and decanting twice with 500 ml 75% acetone and twice with 500 ml dry acetone. The glycerol pectate was then filtered, rinsed on the funnel with 500 ml acetone and dried at 70°C. for two hours. Yield: 64 grams glycerol pectate, Lot #1.

Degree of esterification: - 90% (0.522 meq/g of unesterified COOH groups)

Relative viscosity at 25.0°C.:

<u>Concentration</u>	<u>Solvent</u>	<u>η_{rel}</u>
4%	water	8.45
2%	saline	3.04

Oncotic pressure (P) of a 2% solution in saline against water at 22°C:

<u>Time Hrs.</u>	<u>Pressure mm H₂O</u>
24	135
48	184
72	237
96	274

B. Sorbitol Pectate

In the course of the studies on pectate esters for use as plasma extenders the preparation of sorbitol pectate was also considered. The high number of hydroxyl groups of sorbitol appeared to be of particular interest in incorporating increased water solubility to a pectate ester. Studies along this line were also encouraged by the availability of an inexpensive, commercial starting material. Arlex, manufactured by Altax Powder Company, is an aqueous concentrate consisting of about equal amounts of sorbitol and sorbitol anhydrides. It was hoped to achieve esterification of pectic acid with sorbitol anhydride contained

in Arlex in analogous manner as previously established on oxides of lower polyhydric alcohols.

A few preliminary experiments were conducted in sealed bottles by reacting pectic acid with various amounts of Arlex at 60° for several days. Under these conditions, however, esterification could not be observed. Negative results were also obtained in runs with less concentrated charges using water as diluting agent.

The author has consulted and collaborated with Dr. Rene Jennen on various problems relative to this project.